Prostate cancer as a model for tumour immunotherapy

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Abstract | Advances in basic immunology have led to an improved understanding of the interactions between the immune system and tumours, generating renewed interest in approaches that aim to treat cancer immunologically. As clinical and preclinical studies of tumour immunotherapy illustrate several immunological principles, a review of these data is broadly instructive and is particularly timely now that several agents are beginning to show evidence of efficacy. This is especially relevant in the case of prostate cancer, as recent approval of sipuleucel-T by the US Food and Drug Administration marks the first antigen-specific immunotherapy approved for cancer treatment. Although this Review focuses on immunotherapy for prostate cancer, the principles discussed are applicable to many tumour types, and the approaches discussed are highlighted in that context.

Localized disease

In prostate cancer, this usually refers to disease that does not extend beyond the prostate gland itself, which can be treated with radiotherapy, surgery or the removal of androgens.

Recurrent disease

Cancer that has returned following primary therapy. Recurrent prostate cancer can be detected by a rising level of prostate-specific antigen only (biochemical recurrence) or by computerised tomography or bone scans (metastatic disease).

In developed countries, prostate cancer is the most common cancer in men, and it ranks third overall in terms of mortality (behind lung cancer and colon cancer). Localized disease is treated surgically or with radiation therapy or, alternatively, may be monitored closely if the cancer is thought to be of sufficiently low risk. If disease returns after initial surgery or radiation therapy, this recurrent disease can be treated with androgen ablation (chemical castration or surgical castration) or observed until metastatic progression. Metastatic prostate cancer is initially treated with androgen ablation, but most patients eventually become refractory to this treatment, developing castration-resistant disease, for which the primary treatment option is chemotherapy. This paucity of therapeutic options, as well as their associated morbidity, has led to a search for new treatments; immunotherapy, in which the patients’ immune system is targeted to induce an antitumour response, is a rapidly evolving treatment option. In many ways, prostate cancer is a typical epithelial adenocarcinoma, so the immunotherapy approaches that are being developed for this disease provide insights that are also applicable to other epithelial cancer types. In this Review, we first briefly discuss the basic biology and natural history of prostate cancer, focusing on issues that relate to immunotherapy. We then outline some of the immunotherapy approaches that have advanced to later stage clinical trials, with an emphasis on the immunological and clinical insights provided by these studies.

Immunological characteristics of prostate cancer

With several notable exceptions, most human cancers develop in immunologically intact hosts. So, the progression of tumours from low-grade, localized disease to metastasis involves an interaction between the tumour cells and the host immune system; here, we focus on what is known regarding that interaction in prostate cancer.

Role of inflammation in the development of prostate cancer

As is the case for most types of cancer, the precise aetiology of prostate cancer is unknown; however, a great deal of literature supports the hypothesis that both genetic and environmental factors are important. Interestingly, human and animal studies indicate that inflammation might have a role in prostate cancer development, as well as in the progression from organ-confined to metastatic disease. Inflammation is also thought to have a role in the development of many other human cancers; well-described examples include gastric, colon and liver cancer. A causal relationship between ongoing inflammation and prostate cancer has yet to be established, but substantial epidemiological evidence indicates that prostate cancer is more common in demographic groups with a greater degree of baseline inflammation. Unfortunately, neither the aetiology nor the precise immunological characteristics of intra-prostatic inflammation are well understood. In terms of adaptive immunity, both CD4+ and CD8+ T cells are present in prostate glands, and the CD4+ T cells include both T helper 17 (T_h17)
and regulatory T (Treg) cell populations. Intraprostatic CD8+ T cells in humans are non-functional and do not upregulate activation markers such as CD69 or CD137 in response to stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. These data are consistent with those obtained using antigen-specific CD8+ T cells isolated from melanoma lesions, as well as with transgenic mouse models of prostate cancer (see below). In terms of immunotherapy, these results indicate that prostate cancer vaccination is targeted at an organ with a pre-existing and complex pattern of inflammation that might be contributing to disease progression.

**Early-stage prostate cancer.** Like most solid tumors, prostate cancer generally progresses through a series of stages, known as clinical states (FIG. 1). In developed countries, many cases of prostate cancer are initially detected by monitoring the levels of prostate-specific antigen (PSA) in the blood (BOX 1). Increased (or changing) levels of PSA prompt a biopsy, and a diagnosis of prostate cancer is based on microscopic evaluation of the biopsy specimen. Diagnosis generally leads to an attempt at local treatment, with either surgery or radiotherapy. For up to 80% of surgically treated men, local treatment is successful in that metastatic disease does not occur within 15 years. When disease does recur, the initial manifestation is often a rising PSA level without radiologically detectable metastases, a clinical state known as biochemical progression. An analogous state occurs in some gastrointestinal cancers, in which an increasing level of carcinoembryonic antigen (CEA) can be detected before progression as determined by radiographic imaging. From an immunological perspective, biochemical recurrence provides a unique opportunity for immunological intervention in patients with cancer, as the many immunosuppressive mechanisms (such as Treg cells, myeloid-derived suppressor cells (MDSCs) and transforming growth factor-β (TGFβ)) associated with an advanced tumour burden are expected to be at a minimum at this stage. However, it is difficult to select appropriate endpoints for clinical trials in patients with biochemically recurrent cancers, as neither PSA nor CEA level is a validated surrogate endpoint acceptable for drug registration (BOX 1). More traditional clinical endpoints, such as the development of overt metastases, might not occur for many years, leading to unacceptably long follow-up times for trials with such endpoints.

**Late-stage prostate cancer.** Prostatic epithelial cells are broadly dependent on androgens for survival; hence men with biochemically recurrent disease can be treated with androgen ablation, through either surgical or chemical castration. Although an overall survival benefit for androgen ablation in men with biochemically recurrent disease is not well supported by large, randomized clinical trials, it is of interest to note that this commonly used therapy has many immunological effects, several of which would be expected to increase the efficacy of cancer immunotherapy. Initial observations in this regard came from a study showing that androgen ablation before prostate cancer surgery resulted in a substantial CD4+ T cell infiltration into the gland, and that the infiltrating cells expressed a restricted pattern of use of the T cell receptor β-chain variable region (Vβ) — such an oligoclonal response is consistent with an antigen-specific response. These findings were well supported by a more recent comprehensive analysis of the post-castration immunological infiltrate in the prostate gland, in which an increased CD8+ T cell infiltrate was noted as well. Using an autochthonous mouse prostate cancer model, we found that androgen ablation decreases CD4+ T cell tolerance to a prostate cancer-associated antigen: adoptively transferred, clonotypic CD4+ T cells could respond to specific vaccination after androgen ablation but not in intact, tumour-bearing mice. Perhaps even more intriguing are reports showing that androgen ablation reverses the thymic involution that normally occurs with aging, resulting in increased output of naive T cells. Taken together, these data strongly support the notion that androgen ablation, through its effects on boosting the prostate-specific immune response, could have an additive effect with immunotherapy, a principle that has been formally evaluated in several clinical trials. Interestingly, the relative timing of androgen ablation and immunotherapy could be crucial; one study showed that applying immunotherapy before castration was more effective than the converse.

Eventually, many men with prostate cancer develop metastatic disease, despite androgen ablation. This disease state is known as metastatic, castration-resistant prostate cancer and is the state in which most immunotherapy approaches have been clinically evaluated. These men have a median survival of ~16 months, allowing the timely completion of trials with a survival endpoint. However, from an immunological perspective,
Box 1 | Prostate-specific antigen

Prostate-specific antigen (PSA) is a glycoprotein with expression mainly confined to the epithelial cells that line prostate glands. Disruption of the normal prostatic architecture owing to inflammation, infection or cancer leads to leakage of PSA into the general circulation, where it can be detected by an enzyme-linked immunosorbent assay (ELISA)-based blood test. In 1986, the US Food and Drug Administration (FDA) approved the use of PSA testing to monitor treatments for prostate cancer and, in 1994, testing of PSA levels was approved by the FDA for disease detection. PSA testing has low sensitivity and specificity for prostate cancer detection, and the value of PSA testing for preventing prostate cancer mortality has been evaluated in two recently published randomized trials, one of which supports testing, whereas the second trial does not. Nevertheless, the mortality rate from prostate cancer has declined slowly over the past decade, and there are some data to indicate that the initiation of this decline coincides with the adoption of PSA testing. The American Urological Association (AUA) recommends PSA screening for well-informed men with an estimated ten-year life expectancy. This qualified screening recommendation is based on the notion that many screened men will be diagnosed with low-risk disease and thus face the therapeutic dilemma of whether to undergo primary therapy. In addition, the five-year survival rate for patients with prostate cancer is nearly 100%, so only men with a predicted lifespan long enough to benefit from intervention should probably undergo a screening PSA test. It is noteworthy that this screening recommendation is not shared by other organizations, most of which recommend against routine screening of asymptomatic men. From an immunological perspective, PSA is a target antigen in several immunotherapeutic approaches for prostate cancer, most notably a poxvirus-based vaccine known as ProstVac VF.

Overall, these human and mouse data support a model whereby evolving tumours result in the proliferation of T cells with an anticancer potential but that, in the absence of some intervention, such cells exist in a non-functional or anergic state.

Antigen-specific immunotherapy

The goal of most approaches to cancer immunotherapy is to activate a population of effector T cells, which can then traffic to evolving tumours and mediate the specific lysis of cancer cells. In antigen-specific approaches, a tumour-associated antigen is directly targeted, either by loading that antigen onto antigen-presenting cells (APCs) ex vivo or by incorporating the antigen into a vaccine vector at a protein or DNA level. Below, we summarize the antigen-specific immunotherapy approaches for prostate cancer that have progressed furthest in clinical trials. Although a large number of target antigens could have potentially been selected for prostate cancer, a great deal of clinical work in this area has focused on PSA as a target, most probably because of its long-standing clinical use as a serum marker for the disease. Other prostate-associated antigens include prostatic acid phosphatase (PAP) and prostate-specific membrane antigen (PSMA; also known as GPCII), which is expressed on the vasculature in several types of cancer. The expression pattern of PSA is nearly ideal for its use as an immunotherapy target; it is expressed fairly exclusively by prostate cancer cells and by non-transformed prostate epithelial cells, making it a specific marker of prostate tissue. Notably, a tumour-specific protein is generally not thought to be necessary because, at the time of vaccine treatment, most men with prostate cancer have undergone primary therapy, and the only remaining PSA-expressing cells would be expected to be tumour cells. A few studies also indicate a role for PSA in the initiation and progression of prostate cancer, making it a potentially functional target as well. In the absence of immunotherapy, however, T cell responses to PSA are difficult to detect in patients with later-stage prostate cancer, which indicates that, similar to the mouse model, some level of pre-existing tolerance might exist.

Surrogate endpoint

A biological marker used in a clinical trial to substitute for a clinically relevant endpoint. Some examples include cholesterol level, which can be a surrogate endpoint for studies aiming to reduce the risk of heart disease, or CD4+ T cell count, which can be a surrogate endpoint for reducing the chance of death from opportunistic infections in patients with HIV.

Autochthonous

Arising spontaneously over time. Mouse tumour models that are autochthonous may more accurately model the natural immune response to cancers, as evolving cancers are recognized by the immune system and induce a tolerogenic state.

TRAMP model

(transgenic adenocarcinoma of the mouse prostate model). A mouse model of prostate cancer in which prostate cancers arise spontaneously because the SV40 large T antigen is expressed in a prostate-restricted manner, downregulating the tumour suppressor molecules P53 and RB locally.
**Poxvirus-based vectors.** Viral vectors have several inherent advantages for immunotherapy — they are straightforward to engineer, they can carry large amounts of genetic material and there is a great deal of clinical experience with poxvirus vectors, such as vaccinia virus, as they were used worldwide in the eradication of smallpox. In vivo, poxvirus vectors most probably infect epithelial cells, a proportion of which undergo cell death. Cellular debris, including encoded antigens, is then taken up by nearby immature APCs, which, when appropriately activated, can present these antigens to CD4+ and CD8+ T cells in a pro-inflammatory context (FIG. 2a). Direct infection of APCs, particularly the Langerhans cells in the skin, is another mechanism by which poxvirus vectors can prime an immune response. For prostate cancer, PSA-targeted vaccinia virus-based immunotherapy has proceeded through several steps, including the incorporation of DNA encoding co-stimulatory molecules (lymphocyte function-associated antigen 3 (LFA-3), CD80 and intercellular adhesion molecule 1 (ICAM1); known as TRIMCOM) into the vaccine, as well as optimization of the MHC class II-binding properties of the vaccine antigen. The main disadvantage of poxvirus-based vectors results from the immunological properties that render poxviruses efficacious vaccine vectors: their propensity to induce a strong antibody response makes homologous prime–boost regimens ineffective, as the antibody response to viral proteins dominates over the desired response to encoded antigen (or antigens). To circumvent this immunological limitation, a semi-heterologous prime–boost strategy involving a vaccinia virus prime followed by an analogous fowlpox virus boost (ProstVac VF–TRIMCOM; Bavarian Nordic) was optimized. The clinical development of this agent has been recently reviewed, and includes several trials in which ProstVac VF was combined with other conventional or experimental agents. Perhaps most relevant to the present discussion, several recent trials of ProstVac VF provide important immunological and clinical insights into cancer immunotherapy, which are described later.

**Sipuleucel-T.** In contrast to the ‘off the shelf’ nature of other immunotherapy agents, sipuleucel-T (Provenge; Dendreon Inc.) is a personalized product that is individually manufactured for each patient with prostate cancer. It is similar in some ways to dendritic cell (DC) vaccines, which have been extensively studied in many tumour types. First, leukopheresis is carried out, and monocytes are enriched in the leukopheresis product through density-gradient centrifugation. These cells are then incubated with the targeted immunogen, a fusion protein linking granulocyte–macrophage colony-stimulating factor (GM-CSF) to PAP, before intravenous administration (FIG. 2b). Once infused, these autologous monocytes are thought to mature into functional APCs and to activate PAP-specific CD4+ and CD8+ T cells in treated patients. These activated T cells are then thought to home to tumour lesions, mediating an antitumour response. In this approach, PAP was chosen as the target antigen based on preclinical studies in a rat model that showed that tolerance to PAP in prostate cancer was not mediated by central deletion of PAP-specific T cells, such that PAP-directed vaccination could induce marked T cell infiltration into the prostate gland. In terms of clinical development of immunotherapies for prostate cancer, this agent has progressed the furthest: three Phase III studies have been completed and US Food and Drug Administration (FDA) approval was granted in April 2010, making sipuleucel-T the first antigen-specific immunotherapy approved for cancer treatment. These Phase III trials provide certain immunological insights, which are discussed further below. It should also be noted that this approach is adaptable to other tumour types by changing the nature of the immunogen — that is, by changing the antigen coupled to GM-CSF in the fusion protein.

**Additional approaches.** An additional antigen-specific approach to cancer immunotherapy involves DNA-based vaccines; in contrast to the above approaches which use viruses and patient monocytes as vectors, DNA can be rapidly and precisely synthesized, making it straightforward to target nearly any selected antigen. The main disadvantage of DNA-based vectors is their low level of immunogenicity relative to the highly immunogenic viral vectors described above. To improve the outcome, pro-inflammatory molecules — such as herpes simplex virus type 1 tegument protein VP22 (to enhance spreading from transfected cells to DCs) or Toll-like receptor (TLR) agonists (to activate APCs) — have been incorporated into DNA-based vaccines, or the vectors have been co-administered with GM-CSF as a nonspecific adjuvant. In this context, GM-CSF is thought to function through the recruitment of APCs, particularly DCs, to the vaccine site. A recent clinical study highlights the potential utility of DNA-based vectors in men with prostate cancer: in a population of men with biochemically recurrent disease given a DNA vaccine encoding PAP, PAP-specific T cell responses were induced, as well as an inhibition of the rate of PSA level increase.

Monoclonal antibodies specific for proteins expressed on the surface of tumour cells are a form of passive immunotherapy, which is in contrast to the above approaches, which are all examples of active immunization. Passive immunotherapy is now commonplace in mainstream clinical oncology, with antibodies specific for CD20 (such as rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec)), human epidermal growth factor receptor 2 (trastuzumab (Herceptin; Genentech/Roche)) and other tumour antigens being widely used. Analogous agents are in earlier stages of development in prostate cancer and focus mainly on PSMA as a target. Interestingly, PSMA is overexpressed on tumour-associated vasculature, as well as on the cell surface of prostate cancer cells, making this agent potentially applicable to other types of cancer. Early clinical trials of a humanized, PSMA-specific antibody (J591; Cornell Weill Medical College) showed impressive tumour targeting, but few objective clinical responses.
Examples of antigen-specific immunotherapy for prostate cancer. 

**a** | The ProstVac VF ‘vaccine’ consists of a DNA plasmid encoding the target antigen, prostate-specific antigen (PSA), and a series of three co-stimulatory molecules (lymphocyte function-associated antigen 3 (LFA3), CD80 and intercellular adhesion molecule 1 (ICAM1)). The plasmid cassette is incorporated into a poxvirus backbone in a ‘packaging’ cell line, giving a final vaccine product. In this approach, a vaccinia virus-based prime is followed by a fowlpox virus-based boost. The viral vectors are injected intradermally, where they probably infect the patient’s epithelial cells. This in turn leads to epithelial cell death, following which the cellular debris (including the target antigen PSA) is taken up by host APCs, presenting PAP peptides to the host immune system in a manner that activates CD4+ T cells. A second potential mechanism for antigen presentation involves direct infection of APCs, including the Langerhans cells in the skin. The incorporation of CD80 into the viral vector facilitates the activation of T cells, through the provision of a co-stimulatory signal for T cell activation. LFA3 and ICAM1 add co-stimulation to facilitate T cell activation.

**b** | Sipuleucel-T immunotherapy is similar to a dendritic cell (DC) vaccine and is based on cells from a patient-derived leukopheresis product. These cells are sent to a central processing facility where monocytes are enriched by density-gradient centrifugation. These monocytes are incubated for 36–44 hours with a specific fusion protein, coupling granulocyte–macrophage colony-stimulating factor (GM-CSF) to the target antigen, in this case prostatic acid phosphatase (PAP). In this approach, GM-CSF targets the fusion protein to immature DCs and enhances subsequent DC maturation. Following incubation, the product is sent to the clinic where it is administered intravenously. Once in the patient, the patient’s immature monocytes are thought to mature to fully competent APCs, presenting PAP peptides to the host immune system in a manner that activates CD4+ T cells.

**c** | J591 is an antigen-specific approach using a humanized monoclonal antibody specific for prostate-specific membrane antigen (PSMA). Although early trials used unlabelled antibody, current trials involve 177Lu-labelled J591, a β-ray emitter with a half-life and path-length favourable for radiotherapy and immunotherapy. Here, the antibody specifically targets the radioactive isotope to the target tissue, where tumour cell death is mediated by irradiation.

CDR, complementarity-determining region; TCR, T cell receptor.
were noted in the patients with advanced tumours who were included in these studies. Similar to monoclonal antibodies developed for the treatment of other types of cancer, the current development of J591 has progressed to a radioisotope-labelled version, with the goal of mediating cancer cell death by localizing a radioactive β-ray emitter close to a patient’s tumour mass (Fig. 2c). Several trials involving Lu-labelled J591 are currently in progress, including studies combining this agent with conventional cancer therapy.

**Polyvalent and non-specific immunotherapy**

The antigen-specific immunotherapy approaches discussed above have a distinct advantage in terms of immune monitoring: T cell responses (if they develop) can be assayed using various conventional technologies such as enzyme-linked immunosorbtent spot (ELISPOT) assay. However, skewing of the immune response, as mediated by a potent monovalent antigen-specific immunotherapy, could theoretically lead to tumour antigen loss, which has been documented in melanoma, although not in prostate cancer. Polyvalent immunotherapy vectors might avoid such a situation by simultaneously inducing an immune response to several tumour-associated antigens.

**Cell-based immunotherapy**

The cell-based immunotherapy known as GVAX (BioSante) is one example of a polyvalent approach to tumour immunotherapy, in which GM-CSF-transduced tumour cells are used as a vaccine. Such cells are injected intradermally; the GM-CSF attracts APCs and T cells to the vaccine site, thereby priming an immune response to tumour antigens (Fig. 3a). Earlier GVAX trials attempted to engineer a vaccine using autologous tumour cells from individual patients, but it was later appreciated that tumour antigens can be cross-presented on patients’ APCs, so further clinical development focused on allogeneic tumour cell lines of a particular cancer type transduced to secrete GM-CSF. This approach has been developed for several types of cancer, including pancreatic, breast, lung, haematological and prostate cancers. Prostate GVAX, for example, includes the androgen-sensitive prostate cancer cell line LNCaP, as well as the castration-resistant prostate cancer cell line PC3 (Fig. 3a), and early phase clinical trials suggested that prostate GVAX could induce new antibodies specific for the cell lines injected. Similar to sipuleucel-T, clinical development of prostate GVAX has advanced to the level of randomized Phase III clinical trials. However, for various reasons, these trials have so far not been successful, providing important lessons regarding both clinical trial design and tumour immunology in humans (see below).

**Immune checkpoint blockade.** In addition to the various immunotherapy approaches described above, recent studies in tumour immunology have focused on the concept of immune checkpoints — a series of molecules that function to limit an ongoing immune response (Fig. 3b). Further along in clinical development among the checkpoint inhibitors are antibodies specific for cytotoxic T lymphocyte antigen 4 (CTLA4) (ipilimumab (MDX-010; Bristol-Myers Squibb/Medarex) and tremelimumab (CP-675206; Pfizer)). The importance of CTLA4 in restraining the immune response was apparent from early mouse studies, in which Ctla4 knockout mice died at ~4–6 weeks of age from a lymphoproliferative disorder. CTLA4 blockade has been evaluated in several malignancies, but the most well-developed data come from trials in patients with melanoma, in which the blocking agent is associated with an approximate 10% objective response rate but, also, (as might have been predicted from the knockout mice) a significant rate (25–35%) of clinically important immune-related toxicity. These data are noteworthy as few significant objective responses have been noted in cancer vaccine trials; these clinical data indicate that blocking immune checkpoints that restrain existing antitumour immune responses might be more effective than inducing a de novo antitumour response through vaccination. These data also support the notion that certain patients with cancer might have a population of tumour-specific T cells that are poised to mediate an antitumour response but that are effectively restrained by CTLA4 expression. Ipilimumab has been evaluated in several Phase I and Phase II trials in patients with prostate cancer, and objective clinical responses and decreases in PSA levels have been described. Based on those data, a Phase III trial comparing ipilimumab with a placebo is currently underway in men with castration-resistant metastatic disease who have not responded to prior chemotherapy (www.ClinicalTrials.gov, identifier: NCT00861614).

Another immunological checkpoint that has been targeted recently in clinical trials is that mediated by the molecule known as programmed cell death 1 (PD1). PD1 was initially identified in a library-based screen of CD8+ T cells undergoing apoptosis. Subsequent work identified the ligand for PD1 as B7-H1 (also known as PD-L1) and showed that the interaction between PD1 and B7-H1 leads to an inhibition of T cell function. In animal studies, PD1 blockade potentiates an antitumour immune response, and PD1-deficient animals develop a degree of strain-specific autoimmunity (albeit with a milder phenotype than Ctla4 knockout mice). Perhaps most importantly, human studies showed that increased expression of B7-H1 was associated with a poor clinical outcome in several tumour types, most notably in renal cell carcinoma. PD1 has been less well studied in prostate cancer, although we have found that the CD8+ T cells that infiltrate the prostate gland in men with cancer seem to express PD1. Similar findings were recently reported in patients with melanoma, suggesting that PD1 expression by tumour-infiltrating lymphocytes might be a common occurrence. A Phase I clinical trial of a fully human monoclonal antibody targeting PD1 (MDX-1106; Bristol-Myers Squibb) has been completed, with interesting results. First, this agent was remarkably well tolerated, with few
Intradermal administration

**a** | In a cell-based immunotherapy approach, allogeneic prostate cancer cell lines specific for a particular cancer type are engineered to secrete granulocyte–macrophage colony-stimulating factor (GM-CSF), which first recruits antigen-presenting cells (APCs), such as dendritic cells (DCs), and T cells (not shown here) to the injection site. The injected vaccine tumour cells undergo necrosis, and cellular debris is taken up by the recruited DCs. Next, the DCs must mature to effectively prime an immune response; GM-CSF secreted by the vaccine cells probably has a role here as well. In the prostate GVAX approach, the injected cancer cells are allogeneic with respect to treated patients, so this immunotherapy relies on cross-presentation to prime a CD8+ T cell antitumour immune response. The prostate cancer cell lines used are LNCaP and PC3, which are androgen-sensitive and castration-resistant prostate cancer cells, respectively.

**b** | The immune checkpoint blockade approach is exemplified by antibodies specific for cytotoxic T lymphocyte antigen 4 (CTLA4) (such as ipilimumab and tremelimumab), which block the immunosuppression mediated by the interaction between CD80 and CD86 (on APCs) and CTLA4 (on CD8+ and CD4+ T cells). A second important immune checkpoint, mediated by the interaction between programmed cell death 1 (PD1) on T cells and its ligand B7-H1 (also known as PDL1) on either APCs or tumour cells, has been the subject of several recent early phase clinical trials. The interaction between lymphocyte activation gene 3 (LAG3) on T cells and MHC class II molecules on APCs is also inhibitory; indeed, CD8+ T cell unresponsiveness may depend on the interaction of several, non-overlapping checkpoints. TCR, T cell receptor.

**Figure 3 | Immunootherapy for prostate cancer not directed towards a single tumour antigen.**

Serious adverse events noted. Second, several objective clinical responses were noted in patients with various types of cancer, which is unusual for a Phase I trial of immunotherapy in a heavily pre-treated patient population. Taken together, these data reinforce the relative importance of immune checkpoint blockade in tumour immunotherapy. In addition, if confirmed in larger studies, it would seem that the benign toxicity profile of PD1 blockade could render it an ideal candidate for future combinatorial trials.
Clinical trials of prostate cancer immunotherapy
Several of the immunotherapy approaches for prostate cancer discussed above have been tested in large clinical trials (TABLE 1); a targeted overview of these trials can give unique insights into immunotherapy that might apply to other solid tumours. In addition, these trials provide interesting data regarding the translation of immunological concepts from the laboratory to a clinical setting.

Survival is the most robust clinical trial endpoint. Similar to the readout in a laboratory experiment, a clinical trial must also have a readout, known as a primary endpoint. Clinical trials in patients with cancer often use some measure of tumour progression as the primary endpoint, quantified by a set of formalized criteria known as RECIST (response evaluation criteria in solid tumours) or World Health Organization (WHO) criteria. It is important to note that the RECIST system was developed in the era of cytotoxic chemotherapy, and so these criteria are based on two implicit assumptions: effective therapies shrink tumours, and tumour shrinkage translates to patient benefit. So, a typical endpoint for an oncology clinical trial might be time to tumour progression (TTP), with progression assayed by RECIST. Indeed the first randomized Phase III trial of sipuleucel-T (the D9901 trial) was designed with a TTP endpoint, as was a recently published randomized Phase II trial of ProstVac VF. A statistically significant difference in TTP was not observed between the active immunotherapy and placebo groups in either case. By contrast, both trials showed a clear and statistically significant difference in overall survival between immunotherapy and placebo groups. This observation is consistent with the results of a recently reported randomized Phase III trial of ipilimumab in patients with metastatic melanoma, for which improved survival rates were observed despite a low rate (11%) of objective clinical responses. The mechanisms behind this discrepancy are not completely understood, but they might involve tumour progression before shrinkage, delayed responses and/or prolonged disease stabilization leading to clinical benefit. Modified versions of RECIST and WHO criteria have been proposed, which use alternative definitions of a response and so might more accurately assess the potential clinical benefits of immunotherapy. However, reliance on overall survival as a clinical trial endpoint means that trial registration for men with prostate cancer is currently limited to patients with castration-resistant metastatic disease, which, given the belief that cancer immunotherapy is probably most efficacious in a minimal residual disease setting, means that such trials might not indicate the true potential of a therapy.

Immunotherapy might be more efficacious with a lower disease burden. Although an inverse relationship between tumour burden and immunotherapy response might seem intuitively obvious, there is some controversy surrounding this idea, as objective clinical responses have been noted in patients with advanced cancers treated with adoptive T cell therapy, as well as in patients with several tumour types treated with checkpoint blockade. Nevertheless, a recently published retrospective study of men with prostate cancer enrolled on a Phase II single-arm trial of ProstVac VF provides some clinical evidence for this concept. Here, a well-established predictive algorithm, the Halabi nomogram, was used to stratify patients into those with predicted survival duration greater than or less than the median at the time of trial enrolment. Interestingly, patients with less advanced disease (those with a Halabi-predicted survival duration greater than the median) seemed to benefit clinically from immunotherapy with ProstVac VF, in that their observed survival was significantly longer than predicted. Conversely, patients with a predicted survival duration less than the median seemed not to have any survival benefit from the immunotherapy under study.

Phase III trials should be based on data from Phase II studies. In contrast to Phase III trials in other medical disciplines, such trials often end in failure in patients with cancer — the agent under study does not produce the clinical benefit that the trial was intended to assay. Several hypotheses have been put forward to explain this issue, but perhaps the simplest concerns Phase II trials, which are usually carried out to more accurately quantify the clinical benefit of an agent or approach before moving to a larger, more expensive Phase III study. Although the positive predictive value of Phase II trials for Phase III outcome is not particularly robust, the negative predictive value is strong: a negative Phase II trial in Oncology clearly predicts a negative Phase III result. This issue is exemplified by the development of GVAX immunotherapy for prostate cancer. Early (Phase II) studies established the safety of GVAX, and immunological correlates (the development of tumour antigen-specific antibodies) were used to select a dosing regimen. However, in these Phase II studies, GVAX was never compared directly with chemotherapy, and it was not administered in sequence with chemotherapy. Nevertheless, two Phase III trials were launched — both of which used a chemotherapy comparator group. In the first trial (VITAL-1), GVAX immunotherapy was directly compared with chemotherapy in men with asymptomatic, castration-resistant prostate cancer, despite the fact that few radiographically detectable responses were noted in the Phase II GVAX studies. A second Phase III trial (VITAL-2) was subsequently initiated to test the hypothesis that the combination of immunotherapy plus chemotherapy would extend survival in men with more advanced (symptomatic, metastatic disease. Although chemotherapy had been shown to be at least additive with immunotherapy in animal studies, no Phase II trials were carried out to verify this result in humans or to explore dose and schedule questions. A planned interim analysis of VITAL-2 showed a greater number of deaths in patients treated with the combination of GVAX plus chemotherapy and the trial was closed. Unfortunately, this imbalance outcome has yet to be explained, by either immunological or clinical mechanisms. These events led to an unplanned interim analysis of the earlier trial (VITAL-1), which showed that the trial was unlikely to meet its primary endpoint.
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<td>Randomized trial comparing ProstVac VF with hormonal therapy in men with non-metastatic CRPC; crossover allowed</td>
<td>42</td>
<td>Completed</td>
<td>Increased ELISPOT assay response to PSA in evaluable patients (n = 8) in ProstVac VF–treated group versus hormonal therapy–treated group</td>
<td>Increased survival in men treated with ProstVac VF followed by hormonal therapy</td>
<td>30, 119</td>
</tr>
<tr>
<td>ProstVac VF</td>
<td>Therion Biologics (rights now owned by Bavarian Nordic)</td>
<td>II</td>
<td>Randomized trial comparing ProstVac VF with placebo in men with asymptomatic CRPC (the TBC–PRO–002 trial)</td>
<td>127</td>
<td>Completed</td>
<td>No humoral responses to PSA</td>
<td>Primary endpoint (TTP) not met; overall survival duration (secondary end point) increased (25.1 versus 16.6 months)</td>
<td>102</td>
</tr>
<tr>
<td>Prostate GVAX</td>
<td>Cell Genesys (rights now owned by BioSante)</td>
<td>I/II</td>
<td>Dose escalation of prostate GVAX in men with metastatic CRPC (the G0029 trial)</td>
<td>80</td>
<td>Completed</td>
<td>Increased antibody response to vaccine cell lines</td>
<td>Well tolerated; defined dose or schedule for subsequent Phase III trials</td>
<td>134</td>
</tr>
<tr>
<td>Prostate GVAX</td>
<td>Cell Genesys (rights now owned by BioSante)</td>
<td>III</td>
<td>Open-label, randomized trial comparing prostate GVAX with docetaxel chemotherapy in men with asymptomatic, metastatic CRPC (the VITAL-1 trial)</td>
<td>621</td>
<td>Closed</td>
<td>ND</td>
<td>Closed after an unplanned interim analysis showed futility</td>
<td>79</td>
</tr>
<tr>
<td>Prostate GVAX</td>
<td>Cell Genesys (rights now owned by BioSante)</td>
<td>III</td>
<td>Open-label, randomized trial comparing prostate GVAX plus docetaxel chemotherapy with docetaxel chemotherapy in men with symptomatic, metastatic CRPC (the VITAL-2 trial)</td>
<td>114 (600 planned)</td>
<td>Halted</td>
<td>ND</td>
<td>Halted after an interim analysis showed imbalance of deaths in combined treatment group (67 versus 47)</td>
<td>135</td>
</tr>
</tbody>
</table>
CRPC, castration-resistant prostate cancer; ELISPOT, enzyme-linked immunosorbent spot; ND, not determined; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; TTP, time to tumour progression.*These studies show the variety of immunotherapy approaches that have been translated from the laboratory to a clinical setting, as well as the various stages of clinical development. Only selected, illustrative trials are shown here; additional trials are discussed in several recent reviews136–140.

### Table 1 (cont.)  Selected clinical trials of immunotherapy for patients with prostate cancer*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Sponsoring corporation or institution</th>
<th>Clinical trial phase</th>
<th>Design or description of trial</th>
<th>Number of subjects</th>
<th>Status</th>
<th>Immune system effects</th>
<th>Clinical results or comments</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab</td>
<td>Bristol-Myers Squibb/Medarex</td>
<td>III</td>
<td>Randomized, placebo-controlled trial comparing low-dose radiation with or without ipilimumab in men with metastatic CRPC previously treated with docetaxel chemotherapy (the CA184-043 trial)</td>
<td>800 Planned</td>
<td>Ongoing</td>
<td>ND</td>
<td>ND</td>
<td><a href="http://www.ClinicalTrials.gov">www.ClinicalTrials.gov</a> identifier: NCT00861614</td>
</tr>
<tr>
<td>$^{177}\text{Lu}}$-$\text{J591}$ (PSMA-specific antibody)</td>
<td>Weill Medical College of Cornell University</td>
<td>I</td>
<td>Single-armed trial of $^{177}\text{Lu}$-labelled PSMA-specific antibody</td>
<td>35</td>
<td>Completed</td>
<td>ND</td>
<td>Dose limiting myelotoxicity; well tolerated; Phase II dose determined</td>
<td>65</td>
</tr>
<tr>
<td>$^{177}\text{Lu}}$-$\text{J591}$ (PSMA-specific antibody)</td>
<td>Weill Medical College of Cornell University</td>
<td>II</td>
<td>Randomized trial comparing hormonal therapy (ketoconazole) with or without $^{177}\text{Lu}$-labelled J591 in men with non-metastatic CRPC</td>
<td>140</td>
<td>Ongoing</td>
<td>ND</td>
<td>ND</td>
<td><a href="http://www.ClinicalTrials.gov">www.ClinicalTrials.gov</a> identifier: NCT00859781</td>
</tr>
<tr>
<td>DNA vaccine (prostatic acid phosphatase)</td>
<td>University of Wisconsin Waisman Clinical Biomanufacturing Facility</td>
<td>I</td>
<td>Testing the safety and tolerability of a new DNA-based immunotherapy platform in men with biochemically recurrent prostate cancer</td>
<td>22</td>
<td>Completed</td>
<td>9 out of 22 patients showed increased T cell proliferation in response to PAP; humoral response to PAP not detected</td>
<td>No significant treatment-related adverse events; increase in PSA doubling times versus baseline</td>
<td>62</td>
</tr>
</tbody>
</table>

Androgen ablation. The immunological effects of androgen ablation are surprising because they involve the thymus, which is generally not thought of as an androgen-sensitive organ25 (BOX 2). In aged mice, androgen ablation seems to result in regeneration of the normally involuted thymus and in the output of new T cells, as assayed by increased numbers of T cell receptor excision circles in the peripheral blood26. Similar effects have been observed in humans. As noted above, androgen ablation before prostate cancer surgery results in the infiltration of CD4+ T cells into the prostate gland, and these cells have an activated phenotype27. Also supporting a pro-immunogenic role for androgen ablation are recent data showing the induction of new antibody specificities in treated patients28,29. So, the notion that androgen ablation might enhance an anti-prostate cancer immune response has a strong scientific basis and has been evaluated in several clinical trials. An early study tested one dose of vaccinia virus–PSA vaccine (ProstVac) in combination with androgen ablation, finding the combination to be

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**T cell receptor excision circles**

DNA episomes that are normally produced during the thymic maturation of T cells, specifically during recombination of the T cell receptor genes.
Androgen ablation — which is usually carried out chemically by administration of a leuteinizing-hormone-releasing hormone (LHRH) agonist such as leuprolide acetate or zoledronic acid — is by far the most common treatment for prostate cancer, in patients with both biochemically recurrent and metastatic disease. Recent studies have documented profound immunological effects of this common pharmacological therapy, all of which would be expected to enhance an antitumour immune response:

- CD4+ T cell infiltration into the prostate gland;
- Increase in CD8+ T cell and macrophage density in the prostate gland;
- Mitigation of CD4+ T cell tolerance to a prostate- and prostate cancer-restricted antigen;
- Reversal of thymic involution in aged mice;
- Increase in thymic output of T cells;
- Enhancement of efficacy of immunotherapy in animal models.

Several groups have attempted to make use of these effects in a clinical trial setting; one notable ongoing trial combines androgen ablation with blockade of the immune checkpoint molecule cytotoxic T lymphocyte antigen 4 (CTLA4) using the monoclonal antibody ipilimumab (MDX-010; Bristol-Myers Squibb/Medarex) in men undergoing surgical resection for prostate cancer (www.ClinicalTrials.gov identifier: NCT00170115).

In summary, there is a strong scientific rationale for immunotherapy and androgen ablation to be combined in patients with hormone-sensitive prostate cancer. Well-tolerated clinical trials involving these agents have demonstrated the enhancement of the antitumour immune response that might be expected from combining androgen ablation with immunotherapy. A recent study combining the combination of ProstVac with ipilimumab resulted in a clinical benefit in men with hormone-sensitive prostate cancer who failed chemotherapy, with complete and partial radiographic responses reported in 32% of patients. Although the incidence of new antibody specificities following radiotherapy might be of increasing interest as specific immunotherapy agents are developed for other types of cancer.

Checkpoint blockade. A new approach to immunotherapy involves the combination of several immunological agents to both prime an antitumour response and prevent the suppression of existing and new responses. In prostate cancer, the first published data come from a study combining a CTLA4-specific antibody (ipilimumab) with GM-CSF in an effort to stimulate an endogenous antitumour immune response. At higher doses of ipilimumab, radiographically detectable antitumour responses were noted; the data strongly suggested a threshold effect, with responses noted at levels of ipilimumab greater than 3 mg per kg. Another relevant concept might be to combine an active, specific immunotherapy with an immune checkpoint-blocking agent. In an early clinical test of this concept, prostate GVAX was combined with ipilimumab in a dose-escalation study. Decreases in PSA levels, as well as radiographically detectable tumour responses, were noted but, as is sometimes the case with ipilimumab, these responses were associated with immune-related adverse events, including hypophysitis. A similar combination study has been carried out with ProstVac and ipilimumab, the results of which are currently pending, and a trial combining pancreatic GVAX with ipilimumab is in early stages of accrual. It should be noted that the incidence of high-grade toxicity of ipilimumab may be a limiting factor in these studies. Should PD1-specific antibodies prove to be better tolerated, but still effective as a checkpoint inhibitor, combination trials with PD1-specific antibodies might be more feasible to design and complete.

In summary, there is a strong scientific rationale for combining different types of immunotherapy, as well as for combining immunotherapy with conventional therapy. In some types of cancer, such as breast cancer, these approaches might include tumour-targeted monoclonal antibodies or targeted drugs such as imatinib for chronic myelogenous leukaemia. But such approaches add complexity to clinical trial design, and issues of dosing and sequence are a notable challenge.
Conclusions

The clinical development of immunotherapy for prostate cancer is an instructive example of translational science, in that immunological approaches pioneered in animal studies have eventually proven to have clinical benefit in human cancers. The challenges involved in assessing clinical benefit in early disease stages have so far prevented using these therapies in patients with a less prominent tumour burden, but the recent FDA approval of sipuleucel-T could facilitate new opportunities for appropriate earlier stage trials. Achieving long-term remission in most treated patients is an ambitious goal for basic and clinical scientists, and probably requires the careful integration of several treatment modalities in rational combination therapy approaches.

10. A recent study supporting the concept that B cells mediate the progression of prostate cancer from an androgen-dependent to a castration-resistant state in a mouse model.
15. Fox, S. B. et al. The number of regulatory T cells in prostate cancer is associated with the androgen receptor and hypoxia-inducible factor (HIF)-1α but not HIF-1α. Prostate 67, 625–629 (2007).
A recent Phase I trial showing how DNA-based vaccines can be used in prostate cancer. This trial is noteworthy because it targeted a population of patients with early-stage disease and because the DNA vector used is flexible in terms of potential antigens targeted.


This study provided the initial immunological and scientific rationale for targeting cancer using a genetically engineered secreted GM-CSF.


References 97 and 98 show that the CDB-7 T cells that infiltrate tumors are likely to express PD1, suggesting that PD1 blockade may have therapeutic relevance.
An interesting mechanistic study, showing that certain cancer treatments may prime an immune response through TLR4 agonists released by dying tumour cells.


